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Apoptosis: it's BAK to VDAC

The voltage-dependent anion channel (VDAC) of the mitochondrial outer membrane (MOM; Mannella & Kinnally, 2008)—also known as mitochondrial porin—has long been implicated in regulating the mitochondrial response to certain cell death stimuli (Galluzzi & Kroemer, 2007). This includes a potential role in MOM permeabilization (MOMP) during apoptosis, which is a necessary step in the release of cytochrome *c*—a critical cofactor for downstream activation of caspases—from the organelle. Although controversial, it is possible that VDAC is a component of the permeability transition pore complex, and therefore its influence could also extend to the inner membrane, regulating membrane potential and ATP production. Among the strongest indications that VDAC has a role in the regulation of cell death are the reported interactions between VDAC and members of the BCL2 family of proteins. BCL2 proteins are important regulators of the mitochondrial pathway to apoptosis (Youle & Strasser, 2008). The multi-BH-domain-containing, pro-apoptotic members BAX and BAK are the essential gateways to MOMP, and it has been suggested that both physically interact with VDAC (Galluzzi & Kroemer, 2007). In particular, Cheng and colleagues (2003) concluded that a minor VDAC isoform—known as VDAC2—acts to physically restrain BAK in the MOM in non-stimulated viable cells. However, death stimuli, including that elicited by truncated BID (tBID)—an activated, pro-apoptotic, BH3-only BCL2 family protein—displaced inactive BAK from VDAC2, promoting BAK oligomerization. In view of this collective evidence, it was a surprise when gene deletion studies suggested that all three *Vdac* gene products are dispensable for mitochondrial-dependent cell death in mouse embryonic fibroblasts (MEFs; Baines *et al* 2007). In addition to necrotic cell death driven by defects in the permeability transition pore—elicited, for example, by oxidative stress or calcium overload—a range of other stresses were also unaffected by the deletion of *Vdac1–Vdac3*.

A compelling study by the Hajnoczky group, published in this issue of *EMBO reports,* now reprises the role of VDAC2 in regulating BAK, but suggests a function for VDAC2 that is diametrically opposed to the antagonism of BAK by VDAC2 reported by Cheng and colleagues (2003): VDAC2 promotes tBID-induced apoptosis by recruiting newly synthesized BAK to mitochondria. What is going on? Can three such divergent conclusions—no role, antagonism and promotion of apoptosis by VDAC2—be reconciled? The answer is probably yes, but the focus is on BAK and tBID.

Reconstitution experiments using purified proteins or peptides and liposomes of known lipid composition have suggested a core pathway to MOMP, defined exclusively by BCL2 family proteins (Kuwana *et al*, 2005; Lovell *et al*, 2008); however, non-BCL2 components are unquestionably layered on top of this pathway

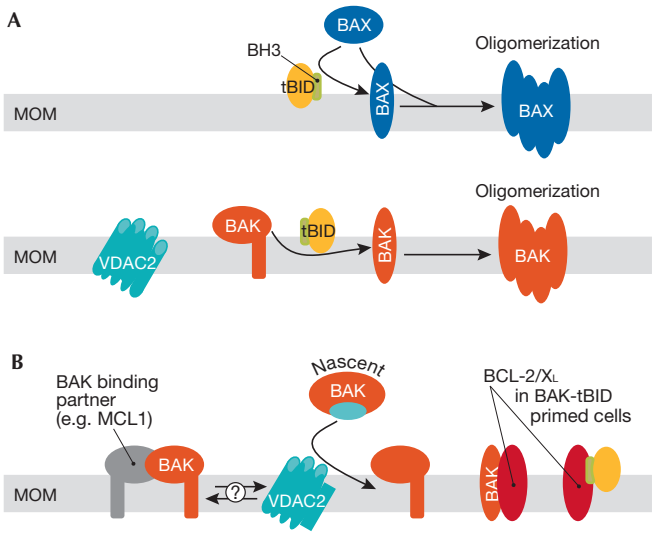

Fig 1 | BAX and BAK at the mitochondrial outer membrane. (**A**) Upper panel: recruitment and activation of BAX at the MOM by tBID (shown in yellow, with its exposed BH3 domain in green). Lower panel: the activation of BAK by tBID probably parallels that of BAX, with the exception that inactive BAK is anchored at the MOM by its TM segment. (**B**) Interaction of nascent BAK with VDAC2 at a presumptive binding site within BAK (shown as a turquoise oval) results in BAK insertion into the MOM through its TM segment. Inactive BAK might then be available for interactions with binding partners, including VDAC2 and MCL1. In cells that express excess BCL2 or BCL-X_L but are primed to undergo apoptosis because tBID has been generated, these pro-survival proteins bind to tBID and activated monomeric BAK, preventing BAK oligomerization and MOM permeabilization. MCL, myeloid cell leukaemia; MOM, mitochondrial outer membrane; tBID, truncated BID; TM, transmembrane; VDAC, voltage-dependent anion channel.

to support its execution and regulation in the complex milieu of the intact cell. The available evidence (Shore & Nguyen, 2008) supports an emerging model that focuses on BAX as the effector of MOMP, and tBID as the 'activator' BH3-only protein (Fig 1A, upper panel). The process starts with the generation of active tBID from inactive BID; tBID then rapidly migrates to the membrane where it probably functions as a receptor for inactive cytosolic BAX, stimulating the conformational transition of BAX to its active, monomeric, membrane-integrated form through the insertion of the transmembrane (TM) segment and integration of helices 5 and 6 into the bilayer, leaving the N terminus available for proteolysis or binding to an antibody. This pathway might be amplified by the ability of activated BAX, similarly to tBID, to recruit more molecules of inactive BAX from the cytosol. Activated BAX can undergo auto-oligomerization, yielding a MOMP-competent complex. In most respects, BAK probably follows a similar process

(Ruffolo & Shore, 2003), with the exception that conformationally inactive BAK is anchored in the membrane through its TM segment (Fig 1A, lower panel), rather than being free in the cytosol. One important consequence of this distinction between BAK and BAX might be that membrane-anchored inactive BAK is available to interact with other proteins that are located in the same membrane.

Irrespective of the membrane-integrated active conformation adopted by monomeric BAX or BAK in the membrane in response to tBID, the TM segment seems to have an essential role, as its deletion typically renders physiologically relevant amounts of ectopic BAX and BAK ineffective in inducing cell death. The TM domain is important for membrane targeting to the resident locations of BAX and BAK, the mitochondria and endoplasmic reticulum. TM segments that confer targeting to the cognate membrane through the signal recognition machinery of the membrane are referred to as 'signal-anchors', whereas TM segments—usually located at either end of a protein—that mediate membrane insertion independently of the membrane signal recognition complex are called 'tail-anchors'. In the case of BAX, the contribution of the membrane signal recognition machinery to mitochondrial protein targeting remains controversial (Ott *et al*, 2009). However, this was specifically addressed for BAK using protein knockdowns, which showed that BAK targets mitochondria independently of mitochondrial protein import receptors (Setoguchi *et al*, 2006). Knockdown of VDAC2, but not of VDAC1, compromised the mitochondrial localization of a BAK construct tagged with three copies of the FLAG epitope at its N terminus and delivered into semi-permeabilized HeLa cells. Conversely, the overexpression of haemagglutinin (HA)-VDAC2—but not of HA-VDAC1—enhanced mitochondrial localization. However, coupling the BAK TM segment to the C terminus of green fluorescent protein (GFP) resulted in efficient targeting of GFP to mitochondria by a VDAC2-independent mechanism, whereas coupling GFP to full-length BAK retained the VDAC2 dependence for mitochondrial targeting of the fusion protein. Collectively, the results suggest that VDAC2 might interact with a remote site on BAK and unmask the TM segment, which would otherwise be unavailable for targeting in the context of full-length BAK (Fig 1B; Setoguchi *et al*, 2006). That is, in the absence of VDAC2, BAK—similarly to BAX—could remain cytosolic in viable cells.

The original suggestion that VDAC2 has a role in the regulation of BAK-mediated cell death (Cheng *et al* 2003) was based on a constitutive interaction between VDAC2 and mitochondrial BAK in viable cells, detected by using a chemical crosslinking approach to identify potential BAK binding partners. The BAK–VDAC2 complex was disrupted by recombinant tBID in isolated mitochondria and after retroviral transduction of tBID into living cells, leading to cell death. Overexpression of VDAC2 inhibited tBID-induced killing of *Bax*$^{-/-}$ MEFs, which express BAK, but not of *Bak*$^{-/-}$ MEFs. Although *Vdac2*$^{-/-}$ MEFs, but not *Vdac1*$^{-/-}$ or *Vdac3*$^{-/-}$ MEFs, showed an enhanced response to stress stimuli, the specific response to tBID was not addressed, nor was it addressed in *Vdac2*-null cells by Baines and colleagues (2007). In the new study by Roy and colleagues (2009), several approaches were used to focus on the tBID pathway, including the delivery of recombinant tBID into semi-permeabilized wild-type and *Vdac2*$^{-/-}$ MEFs, and adenoviral-mediated expression of ectopic tBID in these intact cells. *Vdac2*$^{-/-}$ MEFs showed a clear loss of sensitivity to tBID-mediated mitochondrial depolarization, cytochrome *c* release and apoptosis. Moreover, the expression of VDAC2 rescued tBID sensitivity in *Vdac2*$^{-/-}$ MEFs. Finally, *Vdac2*$^{-/-}$ MEFs also resisted apoptosis initiated by Fas receptor stimulation, a pathway that is mediated by tBID. Thus, VDAC2 seems to have a highly specific role in tBID-mediated apoptosis. Nevertheless, VDAC2 seems to interact with BAK at the mitochondria and this complex can be disrupted by tBID (Cheng *et al* 2003). VDAC2 also has a crucial role in getting BAK to the mitochondrial membrane in the first place (Roy *et al*, 2009). Therefore, BAK is located primarily in the cytosol in *Vdac2*$^{-/-}$ cells, whereas the distribution of tBID remains unaltered. This does not rule out the possibility that VDAC2 supports targeting of BAK to mitochondria and maintains an ability to interact with BAK once it is in the membrane (Fig 1B).

Although the evidence suggests that VDAC2 has a role in targeting nascent BAK to mitochondria, why do *Vdac2*$^{-/-}$ cells resist tBID-mediated apoptosis? BAX would be expected to compensate for the loss of BAK at mitochondria, as cytosolic BAX can be activated by tBID. By using a combination of approaches and cell types with targeted deletions of *Vdac2*, *Bax*, and *Bak*, the results suggest that the BAK–VDAC2–tBID axis is more efficient at initiating apoptosis than the BAX–tBID pathway (Roy *et al*, 2009). Nevertheless, the compounding feature of compensatory changes in cells harbouring targeted deletions of specific genes could be a source of variability between different studies.

In conclusion, an important consequence of VDAC2-mediated targeting of nascent BAK to mitochondria is the spatial separation of mitochondrial BAK and cytosolic BAX, thus opening the possibility for differential regulation of these otherwise redundant effectors of MOMP (Fig 1A,B). In addition to VDAC2, BAK has been reported to interact constitutively with MCL1 in the MOM of viable cells (Cuconati *et al*, 2003; Nijhawan *et al*, 2003). These and potentially other interactions are presumably in equilibrium and can shift to an interaction with tBID depending on the relative protein abundance and interaction affinities (Fig 1B). It is likely that mitochondrial BAK follows the same sequence of activation by tBID and antagonism by pro-survival BCL2 members, as described for BAX. Similarly to BAX, tBID induces a conformational activation of monomeric BAK (Ruffolo & Shore, 2003). BCL2 (and presumably BCL-X_L), which can sequester tBID, also selectively interacts with this tBID-activated BAK conformer, preventing its auto-oligomerization and MOMP (Ruffolo & Shore, 2003). Owing to the spatial separation of BAK and BAX in viable cells, however, there are probably different mechanisms to prevent BAX or BAK from initiating MOMP.

REFERENCES

Baines CP, Kaiser RA, Sheiko T, Craigen WJ, Molkentin JD (2007) Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. *Nat Cell Biol* **9:** 550–555

Cheng EH, Sheiko TV, Fisher JK, Craigen WJ, Korsmeyer SJ (2003) VDAC2 inhibits BAK activation and mitochondrial apoptosis. *Science* **301:** 513–517

Cuconati A, Mukherjee C, Perez D, White E (2003) DNA damage response and MCL-1 destruction initiate apoptosis in adenovirus-infected cells. *Genes Dev* **17:** 2922–2932

Galluzzi L, Kroemer G (2007) Mitochondrial apoptosis without VDAC. *Nat Cell Biol* **9:** 487–489

Kuwana T, Bouchier-Hayes L, Chipuk JE, Bonzon C, Sullivan BA, Green DR, Newmeyer DD (2005) BH3 domains of BH3-only proteins differentially regulate Bax-mediated mitochondrial membrane permeabilization both directly and indirectly. *Mol Cell* **17:** 525–535

Lovell JF, Billen LP, Binder S, Shamas-Din A, Fradin C, Leber B, Andrews DW (2008) Membrane binding by tBid initiates an ordered series of events

culminating in membrane permeabilization by Bax. *Cell* **135:** 1074–1084

Mannella CA, Kinnally KW (2008) Reflections on VDAC as a voltage-gated channel and a mitochondrial regulator. *J Bioenerg Biomembr* **40:** 149–155

Nijhawan D, Fang M, Traer E, Zhong Q, Gao W, Du F, Wang X (2003) Elimination of Mcl-1 is required for the initiation of apoptosis following ultraviolet irradiation. *Genes Dev* **17:** 1475–1486

Ott M, Norberg E, Zhivotovsky B, Orrenius S (2009) Mitochondrial targeting of tBid/Bax: a role for the TOM complex? *Cell Death Differ* **16:** 1075–1082

Roy SS, Ehrlich AM, Craigen WJ, Hajnoczky G (2009) VDAC2 is required for truncated BID-induced mitochondrial apoptosis by recruiting BAK to the mitochondria. *EMBO Rep* **10:** 1341–1347

Ruffolo SC, Shore GC (2003) BCL-2 selectively interacts with the BID-induced open conformer of BAK, inhibiting BAK auto-oligomerization. *J Biol Chem* **278:** 25039–25045

Setoguchi K, Otera H, Mihara K (2006) Cytosolic factor- and TOM-independent import of C-tail-anchored mitochondrial outer membrane proteins. *EMBO J* **25:** 5635–5647

Shore GC, Nguyen M (2008) Bcl-2 proteins and apoptosis: choose your partner. *Cell* **135:** 1004–1006

Youle RJ, Strasser A (2008) The BCL-2 protein family: opposing activities that mediate cell death. *Nat Rev Mol Cell Biol* **9:** 47–59

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